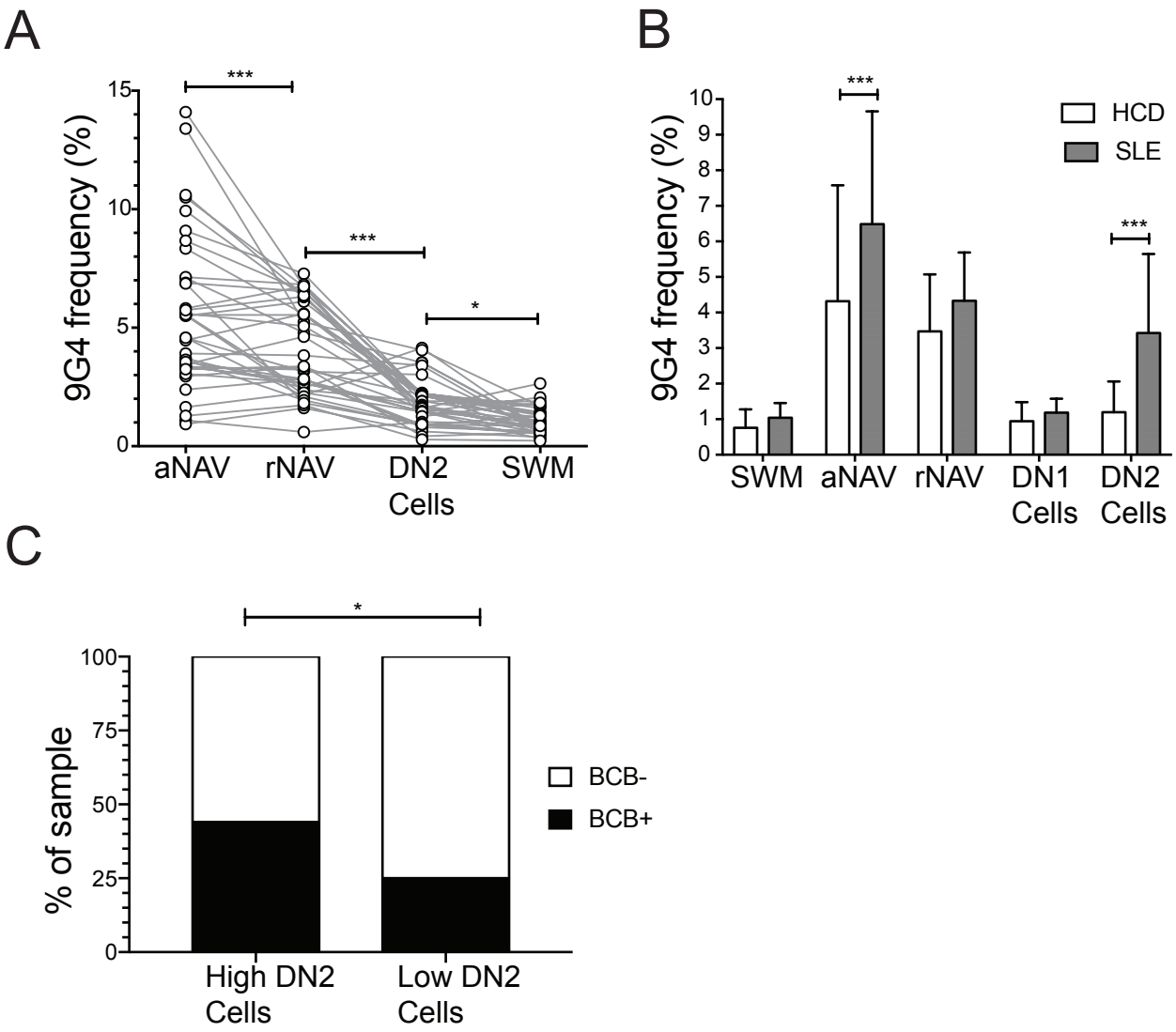
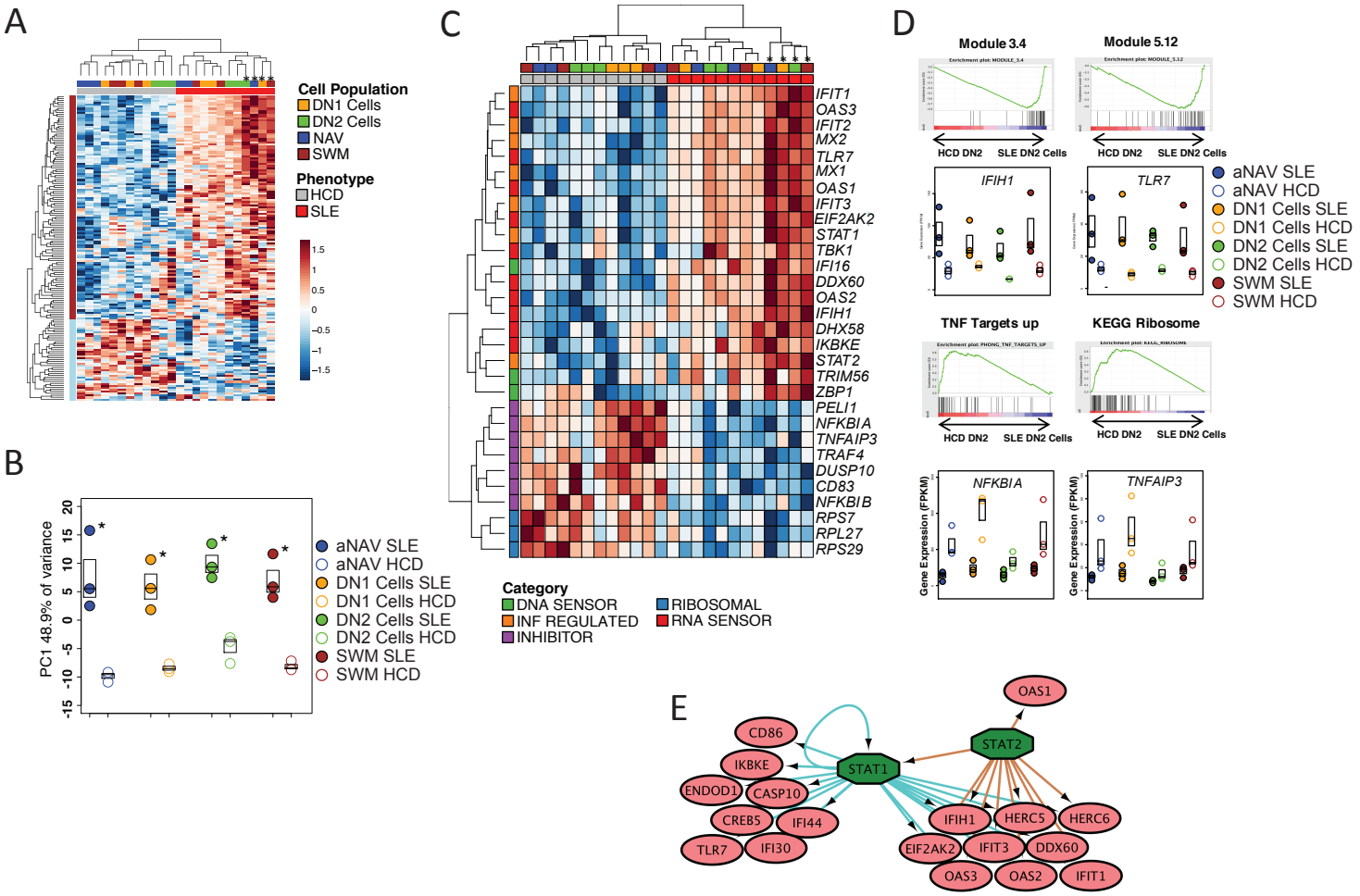
Supplemental Figure 1



Supplemental Figure 1. Elevated frequencies of autoreactive 9G4+ cells and 9G4+ autoantibodies in patients with expanded DN2 cells. (Supporting Figure 3)

A. Frequency of 9G4+ B cells (expressing surface antibody encoded by VH4-34), in different B cell populations from 38 lupus patients. Patients with high-levels of 9G4+ anti-B cell autoantibodies were specifically excluded from this analysis because of interference with the detection of expressed surface 9G4+ BCR. This exclusion leads to under-estimation of the frequency of 9G4+ B cells but ensures high-specificity of the assay (Welch's t-test).
B. aNAV and DN2 cells from SLE patients (n=38) have a higher frequency of 9G4+ B cells than HCD (n=20) aNAV and DN2 cells (Welch's t-test).
C. The frequency of anti-B cell 9G4 serum autoantibodies (black) in patients with a high (H, n=34) and low (L, n=84) DN2 cell frequency (Fischer's exact test).

Supplemental Figure 2



Supplemental Figure 2. B cells from SLE patients express high levels of interferon regulated genes and low levels of negative regulators of innate signaling. (Supporting Figure 4)

A. Heat map generated from RNA-Seq data to visualize the 154 genes differentially expressed between HCD and SLE samples. Both samples (top) and genes (left) are clustered based on Euclidean distance. B cell subset and disease status are indicated by color on the top of the graph. An asterisk indicates patient SLE-3 with active lupus nephritis.

B. Dimension reduction demonstrating separation by one principal component (PC1) of all B cell subsets between HCD and SLE patients.

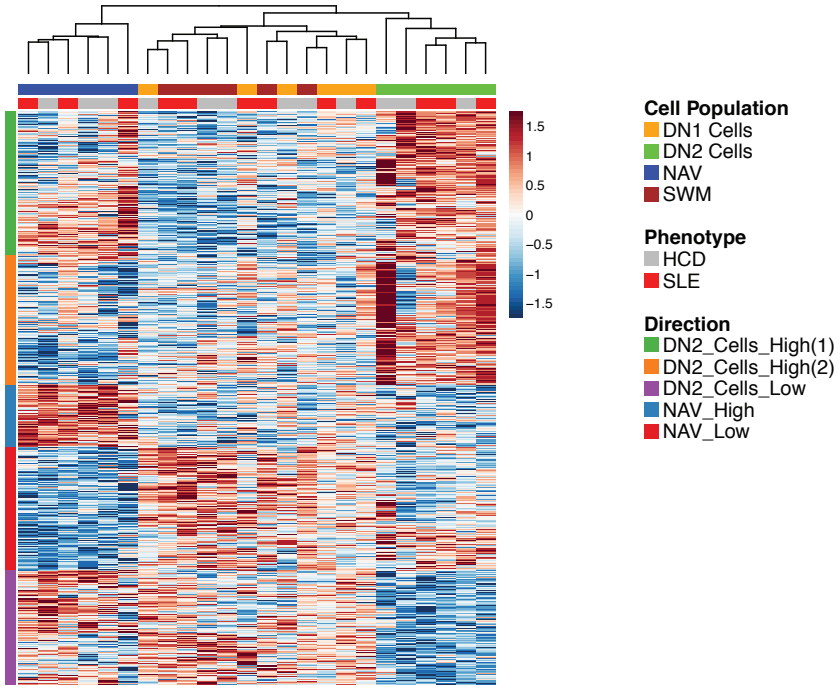
C. Heat map of expression levels for genes grouped by broad functional categories as indicated by the legend. Interferon regulated, RNA sensing pathways, and DNA sensing pathways genes cluster together as they are highly expressed by SLE B cells while negative regulators and ribosomal genes were highly expressed by HCD B cells; Patient SLE 3 is identified by asterisks as before.

D. GSEA plots show preferential enrichment of SLE DN2 cells for interferon regulated genes (top) and lower expression of TNF regulated and ribosomal genes in HCD (bottom). FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values for representative genes in both categories are shown as a dot plot underneath the GSEA plots for all B cell populations in SLE and HCD.

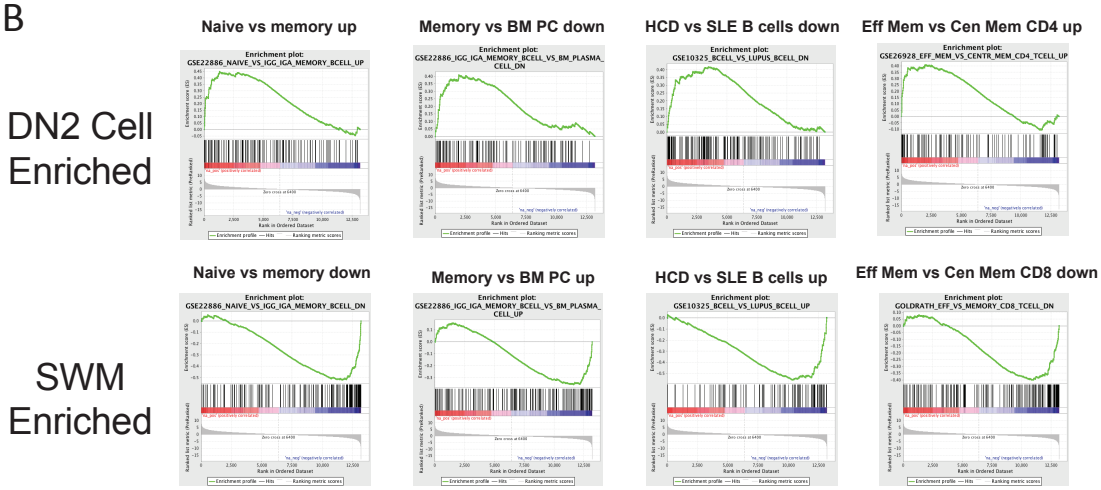
E. Network diagram of select genes up regulated in SLE that are enriched for STAT1 and STAT2 binding motifs as identified by iRegulon. Transcription factors are indicated by green octagons and their target genes by pink ovals with arrows denoting enriched binding for that transcription factor. STAT1 was a DEG as indicated by the inward pointing arrows.

Supplemental Figure 3

A



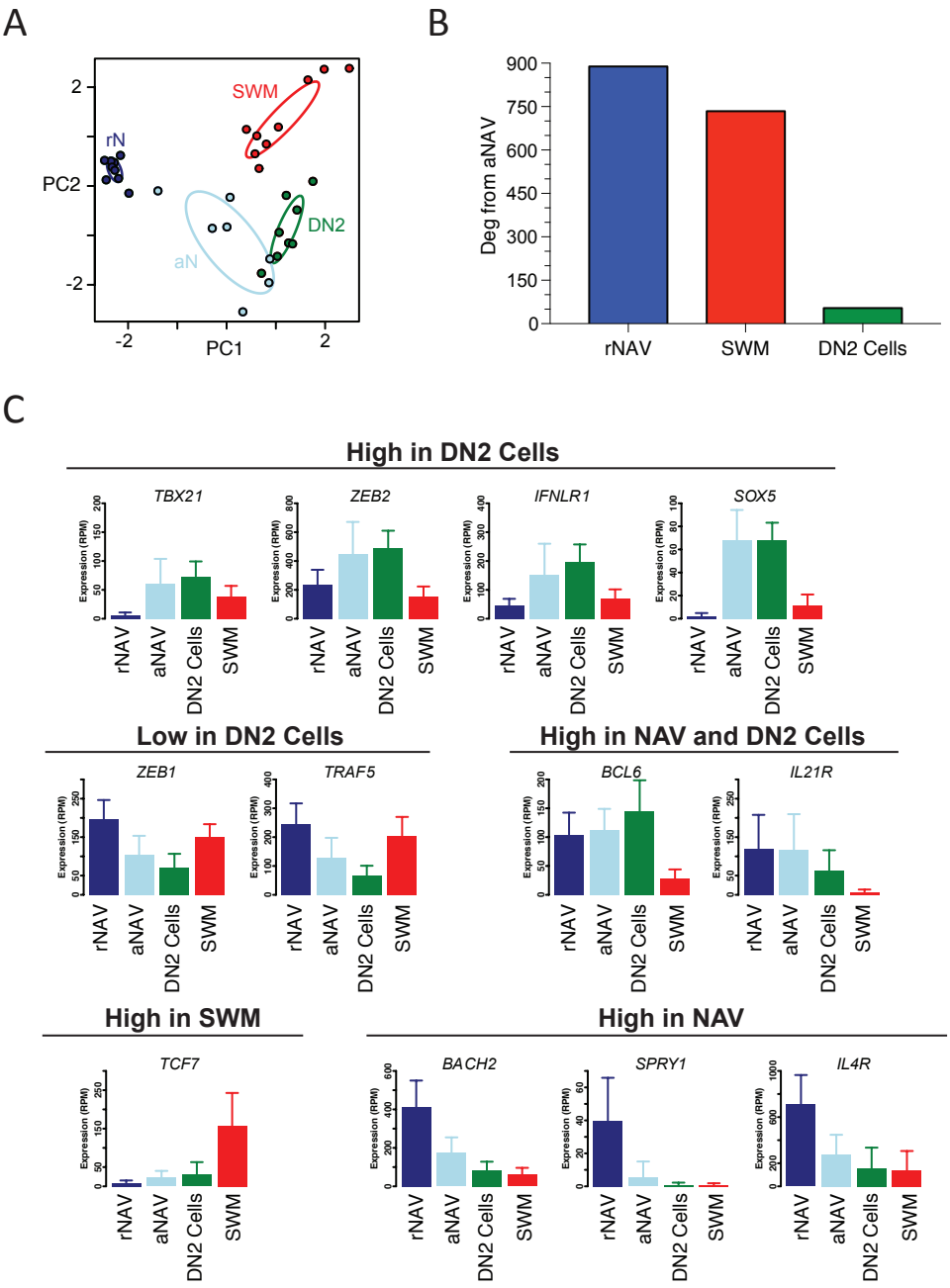
B



Supplemental Figure 3. Global transcriptomic analysis of B cell subsets. (Supporting Figure 4)

A. Heat map of expression of 2,154 genes that are differentially expressed between any two B cell subsets. Both samples (top) and genes (left) are clustered based on Euclidean distance. B cell subset and disease status are indicated by color on the top of the graph, the pattern of expression is indicated by color on the left side.
B. GSEA plots showing reciprocal gene sets enriched in DN2 cells (top) or SWM (bottom). Genes from each gene set are ranked on the vertical axis from most highly expressed in DN2 cells on the left to most highly expressed by SWM on the right.

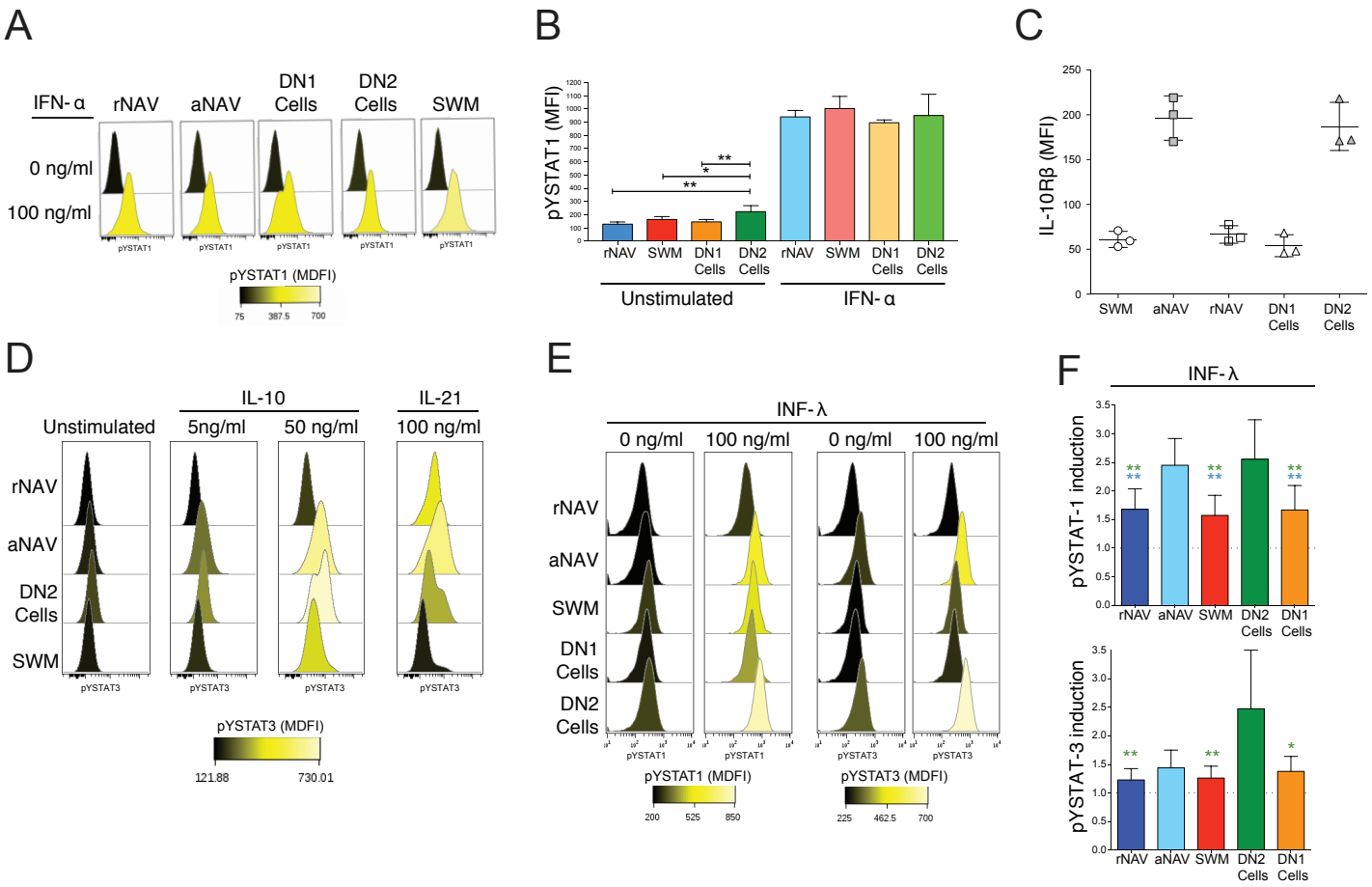
Supplemental Figure 4



Supplemental Figure 4. Activated naïve and DN2 cells are transcriptionally very similar. (Supporting Figure 4)

A. Data reduction of RNA sequencing data from 8 additional SLE patients for activated naïve, resting naïve, DN2 cell and SWM B cell subsets. Circles represent the 95% confidence interval for each B cell subset.
B. Number of differentially expressed genes between aNAV and the indicated B cell subsets.
C. RNA sequencing data for select genes expressed as reads per million mapped reads (RPM).

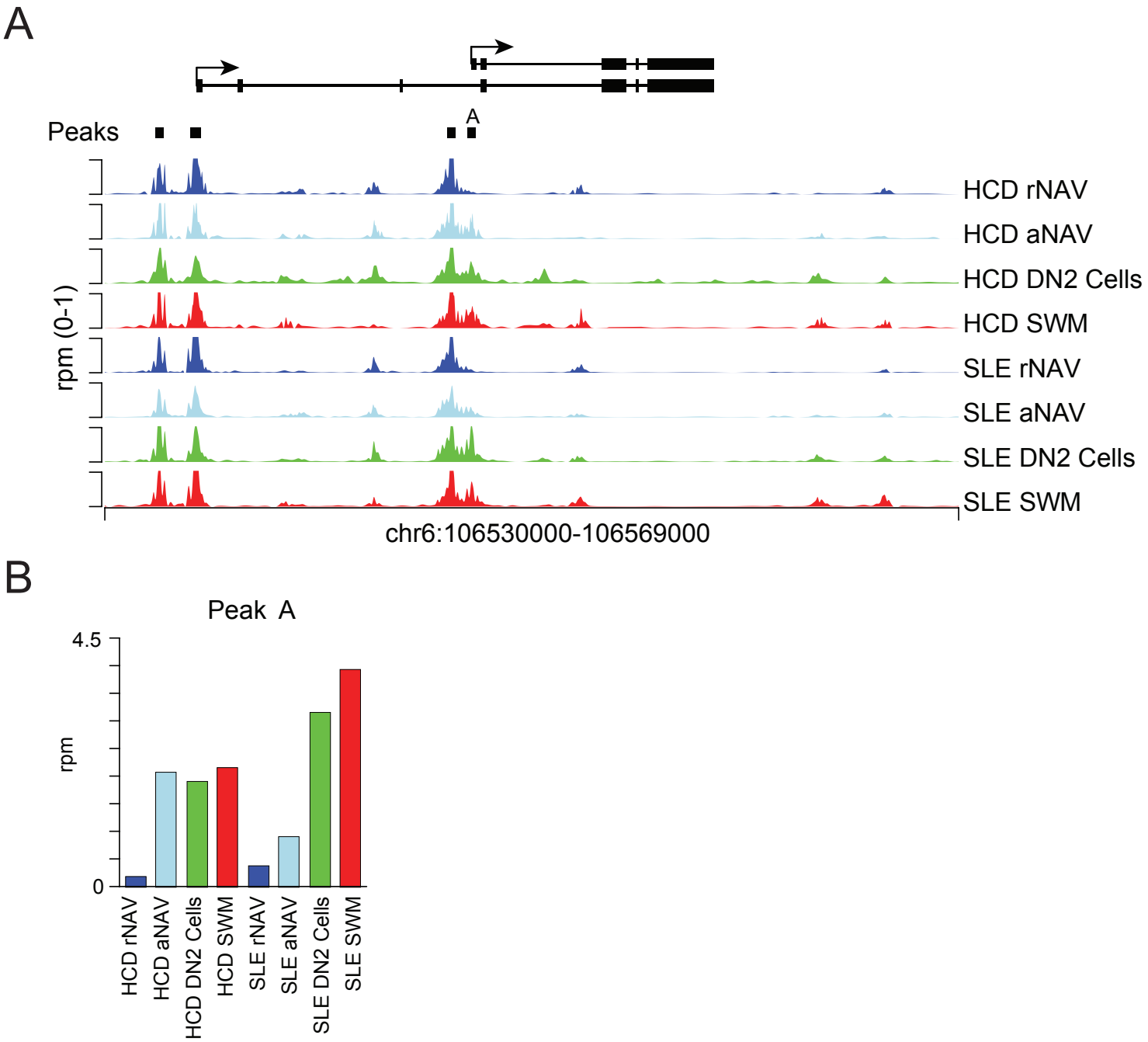
Supplemental Figure 5



Supplemental Figure 5. IL-10, IL-21, and Interferon-l but not Interferon-a signaling is enhanced in DN2 cells. (Supporting Figure 4)

A. Phosphorylated STAT1 staining is shown for either unstimulated or cells stimulated with interferon-a.
B. Median fluorescence intensity of phosphorylated STAT1 staining is shown. Unstimulated DN2 cells have a higher median fluorescence intensity of phosphorylated STAT1 but interferon-a induced phosphorylation does not differ (n=3, mean ± SD, repeated measure 1-way ANOVA).
C. DN2 cells and aNAV express more IL-10RB than other B cells as measured by flow cytometry.
D. IL-10 induced STAT3 phosphorylation is increased in DN2 cells and aNAV relative to other B cells. Only DN2 cells, aNAV, and rNAV respond to IL-21. B cells were stimulated with the indicated dose of IL-10 or IL-21. DN2 cells and aNAV responded at a lower dose of IL-10 and to greater extent as indicated median fluorescence intensity which is shown by color. rNAV, aNAV and DN2 cells phosphorylate STAT3 in response to IL-21 while SWM do not.
E. DN2 cells and aNAV have higher interferon lambda 1 induced STAT1 (left) and STAT3 (right) phosphorylation.
F. INF-l signaling as measured by induced phosphorylation relative to unstimulated cells is significantly higher in DN2 cells and aNAV for STAT1 and significantly higher in DN2 cells for STAT3 and (green*= p vs aNAV, blue*= p vs DN2 cells, n=4, mean ± SD, repeated measure 1-way ANOVA).

Supplemental Figure 6

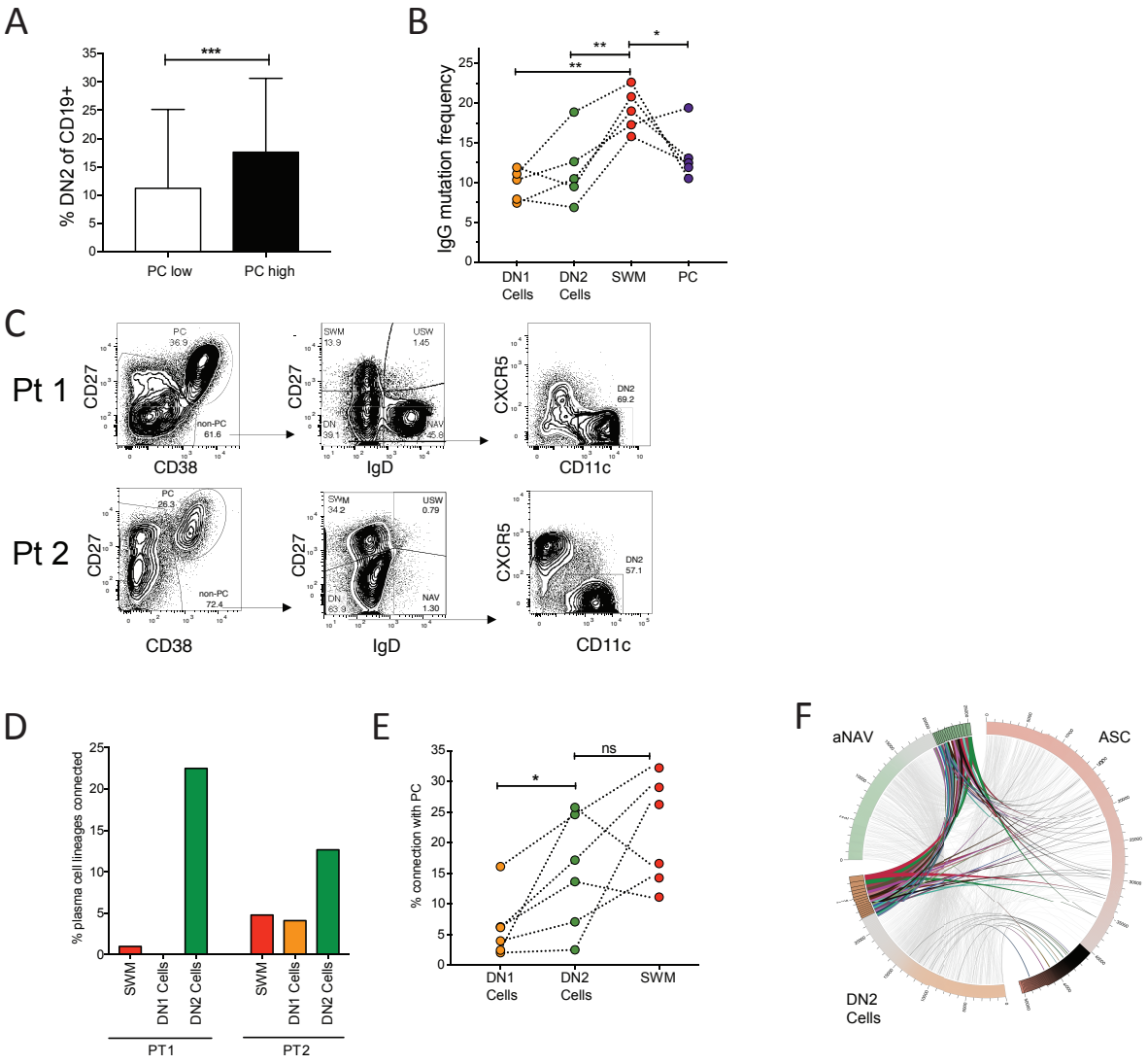


Supplemental Fig 6. Increased chromatin accessibility at the *PRDM1* locus in DN2 B cells. (Supporting Figure 5)

A. Genome plot focused on the gene body and promoter of *PRDM1*. The accessibility of the indicated cell types is shown for HCD and SLE subjects normalized to reads per million (rpm). The locations of peak are denoted by a black bar.

B. Summary of read depth for promoter element peak A.

Supplemental Figure 7



Supplemental Figure 7. The DN2 cell repertoire is highly connected to the plasma cell repertoire in patients with large expansions of both populations. (Supporting Figure 5)

A. SLE patients with expanded plasma cells (n=35) have a higher frequency of DN2 cells than patients without expanded plasma cells (n=114) (Mann-Whitney).
B. Both DN2 cells and antibody secreting cells (ASC) have a lower somatic hypermutation rate than SWM as measured by Next Generation Sequencing of sorted populations. The mean frequency of mutations for IgG heavy chains is shown for each B cell subset for 5 different patients (n=5, repeated measure 1 way ANOVA).
C. Flow profile of 2 patients with large expansion of CD38++ CD27++ plasma cells and DN2 cells.
D. Percentage of plasma cell IgG clonal lineages shared with DN2 cells, DN1 cells and SWM for the two patients showed in B, patient 1 was sequenced by emulsion PCR on single cells (30,000 PC, 70,000 DN2 cells) and patient 2 bulk sequencing.
E. Percentage of plasma cell clonal lineages shared (connections) with DN2 cells, DN1 and SWM for 7 additional patients (repeated measure 1 way ANOVA).
F. Circos plot showing the clonal connectivity between the heavy V-gene of DN2 cells, aNAV and freshly isolated antibody secreting cells (ASC; CD27++CD38++) from an SLE patient. Within each population, sequences are clustered into clones based on same V, J, CDR3 length and 85% CDR3 similarity, and then plotted in size-ranked order with the largest clones being present in the most clockwise position of each population. Number of sequences are identified in the outer ring, and the highlighted portion in each population indicates the D20 fraction (top 20% of all sequences). Connecting lines in the inner space identify shared clonal sequences.

Supplemental Table 1

	Cohort 1	Cohort 2	Healthy
Location	Rochester, NY Baltimore, MD	Atlanta, GA	Rochester, NY Atlanta, GA
Male	7%	8%	24%
Female	93%	92%	76%
Age [1]	42(24-82)	41(23-67)	35 (24-51)
SLEDAI[2]	4(0-12)	5(0-22)	
Ancestry[3]			
AA	37%	92%	24%
EA	47%	6%	71%
Other	15%	2%	3%
Treatment [4]			
PDN	65%	62%	
MMF	35%	29%	
HCQ	78%	76%	
AZA	10%	26%	
CYC	3%	2%	

Supplemental Table 1. Patient Demographics (Supporting Figure 1)

[1] Mean and range
[2] Median and range
[3] AA, African American; EA, European American
[4] PDN, Prednisone; MMF; mycophenolate mofetil, HCQ, hydroxychloroquine; AZA, azathioprine; CYC, cyclophosphamide

Supplemental Table 2

SLE Cohort 1 panel			
Antigen	**Fluorochrome**	**Clone**	**Supplier**
CXCR3	PE	1C6/CXCR3	BD Bioscience
CXCR4	PE-Cy5	12G5	Ebioscience
CD138	PerCP-Cy5.5	MI15	BD Bioscience
CD38	PE-Cy7	HIT2	Ebioscience
CD3	Pacific Orange	UCHT1	Invitrogen
CD14	Pacific Orange	TUK4	Invitrogen
CD27	Qdot605	CLB-27/1	Invitrogen
CXCR5	APC	51505	R&D
IgD	Biotin	IA6-2	BD Bioscience
CD19	APC-Cy7	SJ25C1	BD Bioscience
KI67	FITC	B56	BD Bioscience
VH4.34	PacificBlue	9G4	Custom
Streptavidin	Alexa680		Molecular Probes
Aqua L/D			Molecular Probes

SLE Cohort 2 panel			
Antigen	**Fluorochrome**	**Clone**	**Supplier**
CD138	APC	B-B4	Miltenyi Biotech
CD19	APC-Cy7	SJ25C1	BD Bioscience
IgD	FITC	IA6-2	BD Bioscience
CD3	PerCP-Cy5.5	SP34-2	BD Bioscience
VH4.34	PacificBlue	9G4	Custom
CD27	BV605	L128	BD Bioscience
CD11c	PE	B-ly6	BD Bioscience
CD24	PE-AF610	SN3	Invitrogen
CD21	PE-Cy5	B-ly4	BD Bioscience
CD38	PE-Cy7	HIT2	Ebioscience

Phosphoflow Antibodies			
Antigen	**Fluorochrome**	**Clone**	**Supplier**
BLNK pY84	AF647	J117-1278	BD Bioscience
ERK1/2 pT202/pY204	AF647	20A	BD Bioscience
MAPKp38 pT180/pY182	AF488	36/p38	BD Bioscience

Supplemental Table 2, continued

STAT3 pY705	AF647	4P-STAT3	BD Bioscience
STAT1 pY701	AF488	4A	BD Bioscience
CD20	BV421	H1	BD Bioscience
CD27	BV711	L128	BD Bioscience
CD3	PerCP-Cy5.5	SP34-2	BD Bioscience
CD11c	PE	B-ly6	Biolegend
IgD	Biotin	Goat anti-human	Southern Biotech
IgA	FITC	Goat anti-human	Southern Biotech
Streptavidn	Pacific Orange		Southern Biotech

Other Antibodies			
Antigen	**Fluorochrome**	**Clone**	**Supplier**
IRF4	AF488	IRF4-3E4	Biolegend
IRF8	PE	V3GYWCH	Ebioscience
T-bet	AF647	4B10	Biolegend
SLAMF-7	PE	162.1	Biolegend
FCRL4	PE	413D12	Biolegend
FCRL5	PE	509f6	Biolegend
HLA-DR	BUV395	G46-6	BD Bioscience
CD86	PE	2331(FUN-1)	BD Bioscience
CD22	FITC	HIB22	BD Bioscience
CD32B	APC	4F5	
CD62L	PE	DREG-56	BD Bioscience
CD72	FITC	J4-117	BD Bioscience
IgD	BUV395	IA6-2	BD Bioscience
IgM	FITC	Goat anti-human	Southern Biotech
IgA	PE	Goat anti-human	Southern Biotech
IgG	BUV737	G18-145	BD Bioscience

Supplemental Table 2. Antibody panels used for flow cytometry (Supports Figures 1,3,4,5,and 6)